Effects of increasing ruminally degraded nitrogen and abomasal casein infusion on net portal flux of nutrients in yearling heifers consuming a high-grain diet

C. R. Krehbiel and C. L. Ferrell

J Anim Sci 1999. 77:1295-1305.

Effects of Increasing Ruminally Degraded Nitrogen and Abomasal Casein Infusion on Net Portal Flux of Nutrients in Yearling Heifers Consuming a High-Grain Diet[1,2]

C. R. Krehbiel[3] and C. L. Ferrell

Roman L. Hruska U.S. Meat Animal Research Center, ARS, USDA, Clay Center, NE 68933

ABSTRACT: Seven Meat Animal Research Center (MARC) III heifers (410 ± 25 kg) fitted with hepatic portal, mesenteric venous, carotid catheters, and an abomasal cannula were used in a 7 × 5 incomplete Latin square design experiment. The objective was to evaluate the effects of increasing levels of ruminally degradable N (RDN) with or without the addition of abomasally infused casein on portal-drained visceral (PDV) flux of nutrients. Treatments consisted of dietary CP percentage levels of 9.5 (control), control plus .72% dietary urea (11.5U), control plus 1.44% dietary urea (13.5U), control plus abomasally infused casein (250 g/d; 11.5C), or control plus .72% dietary urea and abomasally infused casein (250 g/d; 13.5UC). All diets contained (DM basis) 80% ground corn, 15% corn silage, and 5% dry supplement and were provided for ad libitum consumption. Nitrogen intake increased (linear, $P < .001$) as CP increased from 9.5 to 13.5%. Portal-drained visceral release of ammonia N increased (linear, $P < .10$) as RDN increased, and was greater ($P < .05$) when protein was fed compared with heifers fed control ($P < .10$). Urea N removal by PDV was not affected ($P > .10$) by level of RDN but was greatest when 11.5C was fed and least when 13.5UC was fed. Net α-amino N (AAN) release by PDV was greatest when 13.5UC was fed (309 mmol/h), least when 9.5% CP was fed (112 mmol/h), and intermediate for the other groups (205 to 252 mmol/h). These data suggest that removal of N by the PDV may promote microbial protein synthesis when dietary RDN is low. When RDN needs have been met and amino acids are deficient for the host, escape protein should be fed to increase amino acid absorption.

Key Words: Feedlots, Nitrogen, Protein

 J. Anim. Sci. 1999. 77:1295–1305

Introduction

Optimizing N utilization in feedlot cattle is essential for maximizing profits and minimizing N waste. Bierman et al. (1996) showed that greater than 89% of the N consumed by feedlot cattle is excreted. This loss of N is an efficiency constraint in feedlot cattle production and potentially contributes to contamination of the environment. Conserving N by optimizing urea recycling to the rumen and minimizing urea excretion in urine and feces may have its greatest benefits in the feedlot industry, because N recycling increases in cattle fed a high-energy, low-protein diet (Huntington, 1989). Therefore, the feedlot industry, which practices feeding cattle 85 to 97.5% concentrate diets, may minimize N waste by optimizing the amount of ruminally degraded N (**RDN**) for microbial protein synthesis and metabolizable amino acids for the host.

Even though numerous experiments have evaluated the effects of urea in finishing diets, to our knowledge no experiments have evaluated the effects of increasing urea with or without escape protein on portal-drained visceral (**PDV**) flux of nutrients in cattle fed high-grain diets. By knowing net flux of nutrients across the PDV when different amounts of RDN and escape protein are administered, the mechanisms by which they influence gut metabolism and peripheral supply of nutrients can be evaluated. This experiment was conducted to compare the effects of increasing RDN with or without the addition of a postruminal supply of casein on intake and net PDV flux of nutrients.

[1]The authors acknowledge Cindy Felber, Chris Haussler, and Jeff Waechter for technical assistance; Randy Kucera, Larry Meyer, and Kathy Sorensen for animal care; and S. Hansen and D. Dill for manuscript preparation.

[2]Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warrant by the U.S. Department of Agriculture and does not imply approval to the exclusion of other products that may be suitable.

[3]To whom correspondence should be addressed. Present address: Dept. of Anim. and Range Sci., New Mexico State Univ., Las Cruces 88003 (E-mail: ckrehbie@nmsu.edu).

Received July 24, 1998.

Accepted November 16, 1998.

Table 1. Composition of diets (% DM basis)

Item	Treatment[a]				
	9.5	11.5U	13.5U	11.5C	13.5UC
Ingredient					
Corn, ground	82.94	82.22	81.50	82.94	82.22
Corn silage	15.00	15.00	15.00	15.00	15.00
Potassium chloride	.86	.87	.87	.86	.87
Limestone	.76	.75	.74	.76	.75
Urea		.72	1.44		.72
Sodium chloride	.30	.30	.30	.30	.30
Trace mineral[b]	.10	.10	.10	.10	.10
Vitamin premix[c]	.01	.01	.01	.01	.01
Rumensin premix[d]	.021	.021	.021	.021	.021
Tylan premix[e]	.013	.013	.013	.013	.013
Casein infused, g/d				250.00	250.00
Nutrient composition[f]					
NEm, Mcal/kg	2.11	2.09	2.08	2.12	2.10
NEg, Mcal/kg	1.44	1.42	1.41	1.45	1.43
Crude protein, %	9.49	11.50	13.50	11.50	13.50
RDP, %[g]	4.61	6.70	8.72	4.61	6.70
Calcium, %	.35	.34	.34	.35	.34
Phosphorus, %	.29	.29	.28	.30	.30
Potassium, %	.85	.85	.85	.84	.84

[a]9.5 = 9.5% CP; 11.5U = 11.5% CP supplied with .72% dietary urea; 13.5U = 13.5% CP supplied with 1.44% dietary urea; 11.5C = 11.5% CP supplied with abomasal casein infusion (250 g/d); 13.5UC = 13.5% CP supplied with .72% dietary urea and abomasal casein infusion (250 g/d).
[b]Trace mineral premix consisted of 13% Ca, 12% Zn, 8% Mn, 10% Fe, 1.5% Cu, .2% I, and .1% Co.
[c]Vitamin premix contained 8,800,000 IU/kg of vitamin A; 880,000 IU/kg of vitamin D; and 880 ppm of vitamin E.
[d]132 g of monensin/kg of premix (Elanco Animal Health, Indianapolis, IN).
[e]88 g of tylosin/kg of premix (Elanco Animal Health, Indianapolis, IN).
[f]Based on tabular values (NRC, 1984).
[g]RDP = ruminally degradable protein. Calculated using the level 1 model of NRC (1996).

Materials and Methods

Experimental

Seven Meat Animal Research Center (MARC) III (Angus × Hereford × Pinzgauer × Red Poll) heifers (BW = 410 ± 25 kg at the beginning of the experiment) were surgically fitted with chronic indwelling catheters in the hepatic-portal vein and two mesenteric veins (Huntington et al., 1989). Catheter patency was maintained by filling catheters with a heparinized-saline solution (1,000 U/mL) between sampling periods. The right carotid artery was elevated (McDowell et al., 1966) to provide access to arterial blood. In addition, an infusion cannula was implanted into the pyloric region of the abomasum. Patency of the abomasal infusion cannula was maintained by filling the cannula with mineral oil between infusion periods (Krehbiel et al., 1996). Experimental procedures were conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Consortium, 1988). Protocols were approved by the U.S. Meat Animal Research Center's Animal Care and Use Committee.

Heifers were used in a 7 × 5 incomplete Latin square design experiment consisting of seven heifers and five sampling periods. The experiment began June 5, 1995, and ended August 13, 1995. Heifers were adapted to a high-grain (7.5% roughage) diet. Treatments (Table 1) consisted of dietary CP percentages of 9.5 (control), 11.5 (.72% dietary urea; 11.5U), 13.5 (1.44% dietary urea; 13.5U), 11.5 (250 g/d abomasally infused casein; 11.5C), and 13.5 (.72% dietary urea and 250 g/d abomasally infused casein; 13.5UC). All diets contained 80% ground corn, 15% corn silage, and 5% dry supplement, with urea substituting for corn in diets 11.5U, 13.5U, and 13.5UC. The diets were offered for ad libitum intake in two equal portions daily at 0630 and 1600. Feed samples were collected twice weekly and composited. Orts were removed daily before the 0630 feeding and weighed. Dry matter and N content of feed and orts were determined (AOAC, 1990). This analysis showed that diets formulated to contain 9.5, 11.5, and 13.5% CP contained 9.1, 11.3, and 13.3% CP, respectively.

Periods consisted of 14 d, which included 10 or 11 d for adaptation to the diet and infusion and 3 d of rest. During the 3-d rest, heifers were housed in an open-front shed in individual pens (3.5 m wide × 3.5 m long). During abomasal casein infusion, heifers were housed in individual stalls (1 × 2 m) in a metabolism barn. The barn had continuous lighting and mechanical ventilation. Heifers were trained to this routine

such that feed intake in the metabolism barn and larger pens was similar. Daily infusions of casein were prepared by dissolving 250 g of sodium caseinate (95% CP and 3.8% ash, DM basis; New Zealand Milk Products, Santa Rosa, CA) in 4,300 mL of tap water. Multichannel peristaltic pumps (Gilson Minipuls 2, Middleton, WI, or Harvard Apparatus Model 1217, South Natick, MA) were used to infuse the casein solution at a rate of 3 mL/min. Tap water (3 mL/min) was abomasally infused in heifers not receiving casein infusion.

Sampling

On d 10 (n = 3) and 11 (n = 4), simultaneous arterial and portal blood samples were taken at 0730, 0930, 1130, 1330, and 1530 for determination of blood flow and PDV flux of nutrients and oxygen. Portal blood and plasma flows were determined by the downstream dilution of a primed (15 mL) continuous infusion (.80 mL/min) of *para*-aminohippurate (**PAH**; 10% wt/vol, pH 7.4) infused through a sterile .45-μm filter into a mesenteric venous catheter. Twenty-five milliliters of arterial or portal blood were collected slowly into heparinized syringes beginning 1 h after the priming dose of PAH, transferred to 50-mL centrifuge tubes containing 50 mg of NaF, and immediately placed on ice for transporting to the laboratory. An additional 1 mL of arterial or portal whole blood was anaerobically drawn into 3-mL heparinized syringes and analyzed immediately for hemoglobin and O_2 saturation using a Hemoximeter (Radiometer America, Westlake, OH), L-lactate using a Model 27 YSI (Yellow Springs Instrument, Yellow Springs, OH), and packed cell volume by centrifuging capillary tubes containing blood.

Blood (8 mL) was frozen (–20°C) immediately. Another 15 mL of blood was centrifuged (15,000 × *g*, 20 min at 4°C), and plasma was harvested and then frozen (–20°C). Three milliliters of plasma from each of the five daily samples per heifer were composited (15 mL) and deproteinized by adding 1.5 mL of 5-sulfosalicylic acid (35% wt/vol). This mixture was vortexed and then centrifuged (11,000 × *g*) for 10 min. The supernatant was decanted and frozen (–20°C) for later analyses. On the day of sampling, 2 mL of blood were mixed with 4 mL of H_2O containing heparin (20 U/mL) for analysis of PAH (Harvey and Brothers, 1962), urea-N (Marsh et al., 1965), ammonia-N (Huntington, 1982), and α-amino N (**AAN**; Palmer and Peters, 1969). Arterial and portal plasma samples were assayed for glucose with the glucose oxidase procedure (Gochmann and Schmitz, 1972) and for PAH using standards prepared from the infusion solutions. Blood VFA were separated from frozen and thawed blood by gas chromatography as described by Freetly and Ferrell (1998). Free amino acid concentrations were analyzed in composited deproteinized blood with HPLC using a lithium ion-exchange column (Pickering Laboratories, Mountain View, CA) followed by postcolumn derivatization.

Calculations and Statistics

Plasma and whole blood flow rates through the PDV and net flux of nutrients and oxygen across the PDV were calculated as previously described (Krehbiel et al., 1992). A positive net flux indicates net release of a nutrient, whereas a negative net flux implies net removal of a nutrient from blood by the PDV.

Means were generated for each heifer within each sampling period by averaging all values obtained from each blood sample for a specific nutrient or oxygen. Therefore, net nutrient flux was calculated as daily venous-arterial concentration difference times daily blood flow for each heifer. Data were analyzed as an incomplete Latin square using GLM procedures of SAS (1990). The model included heifer, period, and treatment as main effects tested against residual mean squares. Four contrast comparisons were constructed to evaluate supplementation effects: 1) control vs supplemental protein [i.e., C – ((11.5U + 13.5U + 11.5C + 13.5UC) × .25)]; 2) 11.5U vs 11.5C; 3) 13.5U vs 13.5UC; and 4) 11.5C vs 13.5UC. In addition, sums of squares due to RDN level were used to test for significant linear and quadratic effects. Results were considered significant at the $P < .10$ level.

Results

Loss of catheter patency for one heifer in periods four and five and three additional heifers in period five resulted in the number of observations being 6, 6, 7, 5, and 6 for the control, 11.5U, 13.5U, 11.5C, and 13.5UC treatments, respectively. Therefore, means presented in Tables 2 through 5 are least-squares means. Daily gain and gain/feed did not differ among treatments ($P > .10$; Table 2). Dry matter intake was greater when supplemental protein was fed ($P = .07$) vs control and was greater when 11.5U was fed vs 11.5C ($P = .09$). Feed N intake was greater ($P < .01$) when heifers were fed additional protein compared with control and when RDN was fed compared with casein infused (i.e., 11.5U vs 11.5C and 13.5U vs 13.5UC). As designed, total N intake was similar ($P > .10$) in heifers consuming 11.5U and 11.5C and in heifers consuming 13.5U and 13.5UC.

Arterial concentration of urea N was greater when heifers were fed supplemental protein compared with control ($P < .01$; Table 3). Abomasal casein infusion (250 g/d) in treatments 11.5C and 13.5UC resulted in an increase in arterial urea concentration of 1.39 and 1.33 m*M* above diets of the same CP (i.e., 11.5U and 13.5U, respectively). Increasing RDN and(or) infusing casein abomasally did not affect ($P > .10$) arterial

Table 2. Dry matter and nitrogen intake and performance in heifers fed increasing ruminally degraded nitrogen and(or) infused abomasally with casein

	Treatment[a]						Contrast[b]			
Item	9.5	11.5U	13.5U	11.5C	13.5UC	SEM[c]	1	2	3	4
Daily gain, kg	1.13	1.56	1.40	1.41	1.21	.19	.23	.57	.46	.48
DM intake, kg/d	8.57	9.88	9.62	8.92	9.25	.39	.07	.09	.50	.56
Gain/feed	.122	.154	.140	.146	.126	.018	.36	.78	.59	.47
Feed N intake, g/d[d,e]	122.1	178.7	204.3	130.4	165.3	6.8	<.01	<.01	<.01	<.01
Casein N infused, g/d	0	0	0	37.2	37.2	—	—	—	—	—
Total N intake, g/d[d,e]	122.1	178.7	204.3	167.7	202.5	6.8	<.01	.26	.86	<.01

[a]9.5 = 9.5% CP; 11.5U = 11.5% CP supplied with .72% dietary urea; 13.5U = 13.5% CP supplied with 1.44% dietary urea; 11.5C = 11.5% CP supplied with abomasal casein infusion (250 g/d); 13.5UC = 13.5% CP supplied with .72% dietary urea and abomasal casein infusion (250 g/d).

[b]Single degree of freedom contrasts were: 1) control vs protein [9.5 – ((11.5U + 13.5U + 11.5C + 13.5UC) × .25)]; 2) 11.5U vs 11.5C; 3) 13.5U vs 13.5UC; and 4) 11.5C vs 13.5UC.

[c]Standard error of the least squares means; n = 6 for 9.5, 11.5U, and 13.5UC; n = 7 for 13.5U; and n = 5 for 11.5C.

[d]Linear effect of ruminally degraded N level ($P < .01$).

[e]Quadratic effect of ruminally degraded N level ($P < .10$).

concentrations of ammonia N, acetate, propionate, isobutyrate, isovalerate, valerate, total VFA (**TVFA**), L-lactate, or oxygen. Arterial concentration of AAN was 10% greater ($P = .06$) when 11.5C was fed compared with heifers fed 11.5U. In contrast, arterial glucose was 11% greater ($P = .04$) when heifers were fed 11.5U compared with heifers fed 11.5C. Arterial concentration of butyrate was greater ($P = .06$) when additional protein was provided vs control.

Portal-arterial concentration (**PA**) difference of urea N responded quadratically ($P < .05$) to increasing RDN (Table 3). In addition, concentration difference of urea N across the PDV was 2.5-fold greater ($P < .01$) when 11.5C was fed vs 11.5U, twofold greater ($P = .02$) when 13.5U was fed vs 13.5UC, and threefold greater ($P < .01$) when 11.5C was fed vs 13.5UC. Portal-arterial concentration difference of ammonia N increased (linear, $P < .01$) as RDN increased and was greater ($P < .01$) when heifers were fed supplemental protein than when heifers were fed control. No differences ($P > .10$) were observed in PA difference of ammonia N when 11.5U vs 11.5C or 13.5U vs 13.5UC were fed. Portal-arterial concentration difference of AAN was greater ($P = .02$) when additional protein was fed than control. Numerically, PA difference of AAN was 29 and 30% greater when 11.5C and 13.5UC were fed compared with 11.5U and 13.5U, respectively. Portal-arterial concentration difference of glucose, propionate, isobutyrate, valerate, and oxygen were not affected ($P > .10$) by treatment. Portal-arterial concentration difference of acetate ($P = .03$) and TVFA ($P = .07$) were greater when 11.5C was fed vs 11.5U and responded quadratically ($P < .05$) to increasing RDN. Portal-arterial concentration difference of butyrate ($P < .01$), isovalerate ($P = .07$), and L-lactate ($P = .09$) were greater when protein was fed and butyrate and isovalerate increased linearly ($P < .01$) as RDN increased.

Portal blood flow was not affected ($P > .10$) by RDN and(or) abomasal casein infusion, although, numerically, portal blood flow was lowest when heifers were fed the unsupplemented control (Table 3). Similar to PA difference, net PDV removal of urea N was greater ($P = .02$) when 11.5C was fed vs 11.5U, greater ($P = .02$) when 13.5U was fed vs 13.5UC, and greater ($P < .01$) when 11.5C was fed vs 13.5UC. Net PDV release of ammonia N was greater ($P = .03$) when additional protein was fed vs control, and it increased (linear, $P < .10$) as RDN increased. Net PDV release of AAN responded quadratically ($P < .10$) to increasing RDN, was 125% greater ($P = .01$) when supplemental protein was fed vs control, and tended ($P = .10$) to be greater when 13.5UC was fed than when 13.5U was fed. Increasing RDN and(or)infusing casein abomasally did not affect ($P > .10$) net PDV flux of glucose, acetate, propionate, isovalerate, valerate, TVFA, L-lactate, or oxygen consumption. Net PDV release of isobutyrate was 40% greater ($P = .09$) when 13.5UC was fed compared with 13.5U, and net PDV release of butyrate was nearly threefold greater ($P = .04$) when supplemental protein was provided compared with control.

Arterial concentration, PA difference, and net PDV flux of amino acids are shown in Table 4. Arterial concentrations of most nonessential amino acids (**NEAA**) were not affected ($P > .10$) by increasing RDN and(or)abomasal casein infusion. The exceptions were tyrosine ($P = .06$) and citrulline ($P = .05$), which were greater when supplemental protein was fed vs control, and tyrosine was greater ($P = .04$) when 11.5C was fed vs 11.5U. Arterial concentrations of the branched-chain amino acids, histidine, and total essential amino acids (**EAA**) were greater ($P < .10$) when supplemental protein was fed vs control, and the branched-chain amino acids, total EAA, and total amino acids (**AA**) were greater ($P < .10$) when 11.5C was fed vs 11.5U. Arterial concentration of total EAA was 21% greater when casein was infused compared with the average of the other treatments.

Portal-arterial concentration difference of cysteine, glycine, tyrosine, total NEAA, valine, and histidine were greater ($P < .10$) when 11.5C was fed compared with 11.5U. In addition, total AA tended to be greater ($P = .11$) when 11.5C vs 11.5U was fed. When 13.5UC vs 13.5U was fed, PA difference of serine, glycine, and α-amino-n-butyrate were greater ($P < .10$), and total NEAA tended ($P = .14$) to be greater.

Table 3. Arterial concentrations, portal-arterial concentration differences, and net portal-drained visceral (PDV) flux for metabolites in heifers fed increasing ruminally degraded nitrogen and(or) infused abomasally with casein

	Treatment[a]						Contrast[b]			
Item	9.5	11.5U	13.5U	11.5C	13.5UC	SEM[c]	1	2	3	4
Arterial concentration, m*M*										
Urea N[d]	4.29	5.18	7.00	6.57	8.33	.68	<.01	.19	.16	.11
Ammonia N	.20	.21	.20	.21	.21	.01	.28	.94	.45	.97
α-amino N	3.13	3.21	3.23	3.53	3.32	.11	.13	.06	.57	.21
Glucose	4.13	4.46	4.10	4.01	4.04	.14	.89	.04	.75	.89
Acetate	.532	.652	.819	.664	.768	.126	.21	.95	.76	.58
Propionate	.045	.056	.051	.050	.038	.006	.57	.54	.13	.22
Isobutyrate	.001	.000	.001	.001	.001	.001	.72	.30	.97	.70
Butyrate	.003	.009	.009	.007	.005	.002	.06	.57	.20	.46
Isovalerate	.003	.003	.007	.006	.005	.002	.37	.39	.40	.67
Valerate	.004	.004	.004	.004	.004	.001	.38	.51	.92	.42
TVFA[e]	.587	.724	.890	.733	.821	.128	.19	.96	.68	.65
L-lactate	.41	.40	.39	.40	.34	.03	.41	.89	.16	.11
Oxygen	6.81	6.58	6.93	6.77	6.50	.28	.69	.64	.26	.52
Portal-arterial difference, m*M*										
Urea N[f]	–.054	–.028	–.051	–.070	–.023	.008	.23	<.01	.02	<.01
Ammonia N[d]	.10	.14	.18	.15	.20	.02	<.01	.70	.61	.18
α-amino N	.13	.21	.23	.27	.30	.04	.02	.35	.22	.61
Glucose	–.070	–.075	–.020	–.045	–.001	.038	.42	.61	.70	.45
Acetate[g]	.588	.380	.775	.713	.585	.157	.82	.03	.14	.37
Propionate	.332	.324	.421	.361	.328	.068	.75	.71	.31	.75
Isobutyrate	.009	.009	.011	.009	.013	.001	.32	.97	.30	.07
Butyrate[d]	.018	.036	.059	.055	.046	.008	<.01	.13	.23	.45
Isovalerate[d]	.012	.013	.039	.025	.033	.007	.07	.24	.58	.45
Valerate	.004	.004	.007	.008	.006	.002	.38	.23	.83	.53
TVFA[eg]	.96	.77	1.31	1.17	1.01	.14	.56	.07	.13	.46
L-lactate	.18	.14	.15	.14	.15	.02	.09	.85	.97	.62
Oxygen	–1.65	–1.60	–1.71	–1.76	–1.66	.09	.79	.23	.68	.44
Portal blood flow, L/h	805	1,097	891	970	1,078	115	.13	.47	.24	.54
PDV flux, mmol/h										
Urea N	–45	–39	–49	–67	–24	7	.92	.02	.02	<.01
Ammonia N[h]	79	145	163	135	211	32	.03	.83	.28	.14
α-amino N[g]	112	252	205	241	309	45	.01	.86	.10	.33
Glucose	–57	–59	–20	–51	–18	30	.17	.84	.13	.58
Acetate	559	466	682	693	606	95	.64	.12	.55	.55
Propionate	318	409	381	354	365	93	.59	.69	.89	.94
Isobutyrate	9	11	10	9	14	2	.36	.48	.09	.06
Butyrate	17	47	53	52	49	13	.04	.78	.89	.87
Isovalerate	13	17	32	24	38	7	.10	.53	.52	.18
Valerate	4	6	6	9	6	3	.34	.50	.92	.58
TVFA[e]	920	956	1,163	1,140	1,079	188	.47	.51	.73	.83
L-lactate	146	157	138	138	178	24	.80	.62	.23	.30
Oxygen	–1,370	–1,752	–1,520	–1,698	–1,754	187	.15	.85	.36	.85

[a]9.5 = 9.5% CP; 11.5U = 11.5% CP supplied with .72% dietary urea; 13.5U = 13.5% CP supplied with 1.44% dietary urea; 11.5C = 11.5% CP supplied with abomasal casein infusion (250 g/d); 13.5UC = 13.5% CP supplied with .72% dietary urea and abomasal casein infusion (250 g/d).

[b]Single degree of freedom contrasts were: 1) control vs protein [9.5 – ((11.5U + 13.5U + 11.5C + 13.5UC) × .25)]; 2) 11.5U vs 11.5C; 3) 13.5U vs 13.5UC; and 4) 11.5C vs 13.5UC.

[c]Standard error of the least squares means; n = 6 for 9.5, 11.5U, and 13.5UC; n = 7 for 13.5U; and n = 5 for 11.5C.

[d]Linear effect of ruminally degraded N level ($P < .01$).

[e]TVFA = Total volatile fatty acids.

[f]Quadratic effect of ruminally degraded N level ($P < .05$).

[g]Quadratic effect of ruminally degraded N level ($P < .10$).

[h]Linear effect of ruminally degraded N level ($P < .10$).

Table 4. Arterial concentration, portal-arterial concentration difference, and net portal-drained visceral (PDV) flux of amino acids in heifers fed increasing ruminally degraded nitrogen and(or) infused abomasally with casein

	Treatment[a]						Contrast[b]			
Item	9.5	11.5U	13.5U	11.5C	13.5UC	SEM[c]	1	2	3	4
Arterial concentration, μM										
Aspartate	18	11	13	14	13	4	.25	.70	.94	.92
Asparagine	44	47	52	59	50	6	.23	.22	.82	.37
Alanine	441	432	381	432	354	35	.34	.96	.69	.20
Glutamate	259	245	230	254	224	29	.53	.84	.87	.50
Cysteine	50	50	56	48	44	5	.83	.75	.10	.62
Serine	179	204	182	212	188	15	.29	.74	.77	.32
Glycine	556	705	559	624	550	56	.40	.34	.91	.40
Tyrosine	114	119	131	149	137	9	.06	.04	.63	.39
Ornithine	166	155	194	216	192	27	.44	.14	.96	.56
Citrulline	100	110	118	134	132	11	.05	.15	.33	.93
α-amino-n-butyrate	18	19	16	16	21	4	.99	.60	.44	.47
Total nonessential	1,945	2,098	1,932	2,154	1,904	145	.64	.80	.88	.27
Threonine	120	131	139	150	141	15	.23	.40	.91	.70
Phenylalanine	137	132	140	157	132	10	.74	.11	.54	.11
Methionine	44	50	53	65	35	8	.46	.24	.15	.04
Valine	459	408	533	674	644	43	.03	<.01	.08	.64
Isoleucine	177	181	200	267	249	26	.10	.04	.20	.65
Leucine	320	321	374	477	429	35	.05	<.01	.25	.38
Arginine	174	218	183	223	214	21	.14	.87	.28	.78
Lysine	196	211	224	268	248	25	.13	.14	.50	.61
Histidine	140	155	149	160	152	5	.02	.53	.71	.33
Total essential	1,770	1,806	2,008	2,445	2,242	144	.04	<.01	.24	.37
Total amino acids	3,714	3,903	3,939	4,599	4,148	254	.14	.09	.55	.26
Portal-arterial concentration difference, μM										
Aspartate	–1[d]	1[d]	8	5[d]	1[d]	5	.33	.60	.29	.60
Asparagine	16	26	16	32	34	8	.23	.62	.19	.90
Alanine	76	77	75	138	122	34	.52	.19	.61	.64
Glutamate	–58	–41	–9[d]	–7[d]	–26[d]	24	.18	.35	.62	.62
Cysteine	–1[d]	1[d]	5[d]	10	2[d]	3	.20	.05	.48	.14
Serine	45[d]	38[d]	8[d]	76	79	27	.87	.36	.09	.94
Glycine	40[d]	20[d]	18[d]	109	126	33	.46	.09	.03	.76
Tyrosine	14[d]	5[d]	13[d]	40	35	11	.45	.05	.15	.76
Ornithine	14[d]	7[d]	9[d]	28	17[d]	13	.92	.30	.66	.59
Citrulline	18	9[d]	10	29	26	9	.94	.14	.18	.83
α-amino-n-butyrate	–2[d]	–1[d]	4[d]	5	–3[d]	3	.23	.11	.08	.06
Total nonessential	151[d]	142[d]	167[d]	464	412	118	.28	.09	.14	.78
Threonine[e]	1[d]	13[d]	49	42[d]	35[d]	30	.29	.53	.74	.88
Phenylalanine	18[d]	12[d]	9[d]	40	34	12	.68	.14	.13	.76
Methionine	5[d]	7[d]	6[d]	25	17	8	.34	.14	.37	.48
Valine	17[d]	14[d]	27[d]	94	66	30	.31	.09	.37	.54
Isoleucine	5[d]	28[d]	19[d]	40	40	18	.19	.67	.44	.98
Leucine	16[d]	29	29	82	74	26	.20	.23	.20	.84
Arginine	31[d]	6[d]	14[d]	38	28[d]	21	.68	.31	.62	.75
Lysine	22[d]	24[d]	24[d]	68	55	20	.34	.16	.29	.67
Histidine[e]	6[d]	6[d]	13	34	20	6	.07	<.01	.43	.13
Total essential	110[d]	133[d]	172[d]	455	372	140	.28	.14	.30	.70
Total amino acids	261[d]	274[d]	339[d]	919	784	255	.27	.11	.21	.73
PDV flux, mmol/h										
Aspartate	–2[d]	1[d]	7[d]	5[d]	0[d]	5	.30	.62	.33	.52
Asparagine	15	29	20	31	30	8	.15	.80	.42	.89
Alanine	67	100	67	136	116	32	.33	.31	.68	.49
Glutamate	–44	–40	–11[d]	–7[d]	–28[d]	20	.26	.18	.52	.34
Cysteine	–2[d]	0[d]	5[d]	10	2[d]	3	.25	.06	.52	.11
Serine	42[d]	52	17[d]	76	77	25	.61	.51	.11	.99
Glycine	37[d]	22[d]	15[d]	106	120	29	.39	.07	.02	.76
Tyrosine	15[d]	8[d]	11[d]	39	34	11	.49	.08	.14	.74
Ornithine	13[d]	11[d]	8[d]	30	12[d]	13	.85	.32	.81	.35

(continued)

Table 4 *(continued).* Arterial concentration, portal-arterial concentration difference, and net portal-drained visceral (PDV) flux of amino acids in heifers fed increasing ruminally degraded nitrogen and(or) infused abomasally with casein

Citrulline	16	9[d]	9	29	25	9	.81	.13	.17	.77
α-amino-n-butyrate	–1[d]	–0[d]	3[d]	5	–2[d]	2	.26	.09	.09	.05
Total nonessential	144[d]	195	145[d]	464	386	110	.22	.12	.12	.65
Threonine	2[d]	21[d]	38	39	33[d]	23	.24	.59	.87	.86
Phenylalanine	18	17[d]	6[d]	38	35	12	.63	.25	.08	.89
Methionine	7[d]	10[d]	6[d]	25	16	7	.35	.18	.35	.41
Valine	16[d]	27[d]	25[d]	94	58	26	.23	.09	.37	.37
Isoleucine	4[d]	41	17[d]	38	33	17	.14	.88	.53	.86
Leucine	15[d]	47	25	79	69	23	.14	.42	.16	.79
Arginine	31[d]	13[d]	9[d]	43	11[d]	23	.64	.38	.94	.37
Lysine	20[d]	36	23[d]	68	48	18	.26	.25	.35	.47
Histidine	6[d]	8[d]	13	34	20	5	.04	<.01	.35	.09
Total essential	112[d]	203[d]	139[d]	451	328	126	.25	.21	.28	.53
Total amino acids	256[d]	398	284[d]	916	714	234	.23	.16	.18	.58

[a]9.5 = 9.5% CP; 11.5U = 11.5% CP supplied with .72% dietary urea; 13.5U = 13.5% CP supplied with 1.44% dietary urea; 11.5C = 11.5% CP supplied with abomasal casein infusion (250 g/d); 13.5UC = 13.5% CP supplied with .72% dietary urea and abomasal casein infusion (250 g/d).

[b]Single degree of freedom contrasts were: 1) control vs protein [9.5 – ((11.5U + 13.5U + 11.5C + 13.5UC) × .25)]; 2) 11.5U vs 11.5C; 3) 13.5U vs 13.5UC; and 4) 11.5C vs 13.5UC.

[c]Standard error of the least squares means; n = 6 for 9.5, 11.5U, and 13.5UC; n = 7 for 13.5U; and n = 5 for 11.5C.

[d]Values do not differ from zero ($P > .10$).

[e]Linear effect of ruminally degraded N level ($P < .10$).

In general, net PDV removal or release of these amino acids followed similar trends as PA concentration difference. Portal-arterial concentration difference and net PDV release of total EAA and total AA were numerically greater when casein was infused than when heifers were fed control or urea supplements. Net PDV release of total amino acids was 2.6-fold greater when casein was infused [(11.5C + 13.5UC)/2] compared with the average of the other treatments [(control + 11.5U + 13.5U)/3].

Daily N exchange (g/d) across visceral tissues is summarized in Table 5. Total N exchange across the PDV was greater ($P < .01$) when heifers were fed supplemental protein than when heifers were fed control. Net PDV exchange of total N was 241, 38, 56, and 61% greater when 13.5UC was fed than when control, 11.5U, 13.5U, or 11.5C were fed, respectively. Nitrogen output (i.e., ammonia N + AAN) by the PDV was 47, 70, 56, 66, and 83% of N input (i.e., intake N + urea N removal) when control, 11.5U, 13.5U, 11.5C, or 13.5UC were fed, respectively.

Discussion

General

Our goal was to obtain DMI and ADG similar to that observed in heifers consuming a high-grain diet in the feedlot. Throughout the experiment, daily DMI was 9.25 ± .39 kg (DM basis) and ADG was 1.34 ± .19 kg, which is similar to (Mader et al., 1995) or lower than (Larson et al., 1993) previous data. In addition, ADG was similar to steers of the same composite breed consuming a high-energy diet (Gregory et al., 1991).

Although numerous experiments have evaluated urea in finishing diets, less information is available concerning the mechanisms of response or lack of response to increasing urea. In a recent experiment, Milton et al. (1997) showed that ruminal OM and starch digestion were improved when .5% urea was fed, with little or no additional improvement when 1.0 or 1.5% were fed to steers consuming a 90% concentrate diet. Even though ruminal ammonia concentrations increased linearly with increasing urea, duodenal flow of total and microbial N and microbial efficiency were not greater than observed on the basal diet (Milton et al., 1997). The authors suggested that the addition of urea improved energy utilization by the animal but did not improve metabolizable protein supply to the small intestine. In our experiment, net PDV release of AAN was 125 and 83% greater when 11.5U and 13.5U were fed, respectively, compared with the control. In contrast to the results of Milton et al. (1997), increasing AAN release by the PDV with the addition of urea suggests metabolizable protein supply to the small intestine was increased when .74% urea was fed, with no further improvement when 1.44% urea was fed. The fact that removal of urea N by the PDV was not increased with increasing urea suggests that urea was lost in the urine, resulting in inefficient use of N. Release of total VFA and lactate, removal of glucose, and oxygen consumption by the PDV were not affected by RDN. These data suggest that for cattle consuming a high-grain diet, an optimal

Table 5. Daily exchange of nitrogen (g/d) across the portal-drained viscera in heifers fed increasing ruminally degraded nitrogen and(or) infused abomasally with casein

	Treatment[a]						Contrast[b]			
Item	9.5	11.5U	13.5U	11.5C	13.5UC	SEM[c]	1	2	3	4
Ammonia N[d]	26.5	48.8	54.9	45.2	70.9	10.8	.03	.82	.28	.14
α-amino N[e]	37.7	84.8	68.9	80.8	103.7	15.2	.01	.86	.10	.33
Urea N	−15.2	−12.9	−16.6	−22.5	−7.9	2.4	.92	.02	.02	<.01
Total N[d]	48.9	120.5	107.2	103.4	166.7	22.7	<.01	.62	.07	.08
Intake N[f]	122.1	178.7	204.3	167.7	202.5	6.8	<.01	.26	.86	<.01

[a]9.5 = 9.5% CP; 11.5U = 11.5% CP supplied with .72% dietary urea; 13.5U = 13.5% CP supplied with 1.44% dietary urea; 11.5C = 11.5% CP supplied with abomasal casein infusion (250 g/d); 13.5UC = 13.5% CP supplied with .72% dietary urea and abomasal casein infusion (250 g/d).

[b]Single degree of freedom contrasts were: 1) control vs protein [9.5 − ((11.5U + 13.5U + 11.5C + 13.5UC) × .25)]; 2) 11.5U vs 11.5C; 3) 13.5U vs 13.5UC; and 4) 11.5C vs 13.5UC.

[c]Standard error of the least squares means; n = 6 for 9.5, 11.5U, and 13.5UC, n = 7 for 13.5U, and n = 5 for 11.5C.

[d]Linear effect of ruminally degraded N level ($P < .10$).

[e]Quadratic effect of ruminally degraded N level ($P < .10$).

[f]Linear effect of ruminally degraded N level ($P < .001$).

amount of RDN can be fed that will enhance fermentation in the rumen, increase amino acid flow to the duodenum, and increase net portal appearance of amino acids without affecting energy use by the PDV. When the need for RDN has been met, no further benefit to additional RDN is expected.

Net Removal of Urea N by the PDV

By design, heifers consuming 11.5U and 11.5C received similar amounts of N, although the source and site of N were different. When 11.5C was fed, net PDV removal of urea was 9.6 g/d greater than when 11.5U was fed. The greater removal of urea N by the PDV when 11.5C vs 11.5U was fed may have supported microbial protein synthesis when RDN was low, suggesting that increasing N intake may be more important than increasing RDN intake when dietary CP is low. Similar to these results, Bruckental et al. (1997) found a 26% increase in removal of urea N by the PDV in steers fed a high-grain diet with low protein (10.7%) and infused with 300 g/d of casein compared with steers fed the basal diet with no casein infusion. In addition, Kreikemeier et al. (1994) found that urea N removal by the PDV was 10 mmol/h (33%) greater when sheep were fed feather meal plus blood meal vs urea as protein sources added to a 95% concentrate diet. Interestingly, the Level 1 NRC model (NRC, 1996) predicted that the RDN requirements were 23 and 55 g/d deficient when heifers were fed 11.5U and 11.5C, respectively. Although it should be cautioned that only net changes were measured in our experiment, removal of urea N by the PDV was 12.9 and 22.5 g/d when 11.5U and 11.5C were fed, respectively, accounting for 56 and 41% of these deficiencies. Portal-drained viscera removal of urea N was greater with abomasal casein infusion compared to urea. However, the magnitude of the increase was not great enough to overcome the estimated deficiency in RDN.

Kennedy and Milligan (1980) suggested that transfer of blood urea into the rumen is affected by ruminal ammonia concentration, the amount of OM fermented in the rumen, and blood urea concentration. In our experiment, infusing 250 g/d of casein abomasally in addition to the control diet (11.5C) resulted in removal of urea N by PDV being threefold greater than when .74% urea was fed and casein was abomasally infused (13.5UC). Arterial concentration of urea N tended ($P = .11$) to be greater when 13.5UC was fed than when 11.5C was fed (8.33 vs 6.57 m*M*). Guerino et al. (1991) found that casein infusion increased arterial concentration of urea N, which was related to increased urinary N excretion. Even though not measured in our experiment, the similar or greater arterial urea N and lower urea N removal by the PDV in heifers fed 13.5UC suggests that urinary urea excretion was increased. One could expect urea N recycling to the PDV to be less in heifers fed 13.5UC than 11.5C because the addition of urea with abomasal casein infusion may have increased the N pool in the rumen that was available for fermentation and microbial cell production.

The NRC (1985) predicted that, as a percentage of N intake, recycled N would be 36.8, 26.4, and 18.5% of N intake for cattle fed 9.5, 11.5, and 13.5% CP, respectively. Although N recycling was not directly measured in our experiment, as a percentage of N intake, PDV removal of urea N was 12.4, 7.2, 8.1, 13.4, and 3.9 ± 1.3 for control, 11.5U, 13.5U, 11.5C, and 13.5UC, respectively. Huntington (1986) reported values ranging from 10 to 42%. In steers fed a high-grain diet, Huntington (1989) found that 57% of hepatic urea production was removed by the stomach via the ruminal wall (45%) or saliva (12%) and only 3% was removed by poststomach tissues. In our experiment, the greater removal of urea N when 13.5U was fed compared with 13.5UC at similar arterial urea N concentrations suggests that site of

protein digestion (i.e., ruminal vs postruminal) may affect urea N removal by the PDV. However, simple linear regression indicated that the correlation between urea N removal by the PDV and total N intake (i.e., diet plus casein infusion; $r^2 = .13$) was low and was similar to the correlation between urea N removal by the PDV and N intake (diet only; $r^2 = .13$). These correlations may suggest that site of protein digestion does not affect urea N removal by the PDV.

Recovery of Casein N

When 250 g of casein was infused per day in heifers consuming the control diet (11.5C), AAN net appearance in portal blood was 43.1 g/d greater than when heifers were fed control, resulting in a 116% recovery of casein N infused. Similar to these results, Bruckental et al. (1997) recovered greater than 100% of amino acid N in portal blood when steers consuming a high-grain diet were infused with casein. When 250 g of casein was infused per day in heifers consuming .74% urea (13.5UC), AAN net PDV appearance was 18.9 g greater than in heifers consuming 11.5U, resulting in a 49% recovery of casein N infused. Similar to these results, Guerino et al. (1991) found a 26.1% recovery of casein N infused when 150 g of casein was abomasally infused per day and a 30% recovery of casein N when 300 g of casein was infused per day. Interestingly, in the study of Bruckental et al. (1997), CP in the basal diet was 10.7%, which is below the NRC (1984) requirement for growing/finishing beef cattle, whereas in the study of Guerino et al. (1991), CP in the basal diet was 13.8%, which met the requirement for steers used in their experiment. In these experiments, the greater recovery of casein N when dietary CP was low may suggest that intestinal hydrolysis and(or) amino acid absorption is more efficient when dietary CP is limiting. Alternatively, the greater than 100% recovery may reflect increased endogenous secretions in response to casein, which would provide more amino acids for appearance in portal blood.

Factors that may contribute to low recoveries of casein N in portal blood have been discussed by Guerino et al. (1991) and include use of amino acids by intestinal mucosa, peptide absorption across the PDV, and ammonia formation in the gut. In our experiment, 49.7 and 40.6% of the casein N infused could be accounted for in portal blood as ammonia N when 11.5C and 13.5UC were fed compared with heifers fed control or 11.5U, respectively. These results fall between values reported by Guerino et al. (1991) when 150 or 300 g/d of casein was infused. Recently, MacRae et al. (1997) demonstrated that mesenteric-drained viscera (**MDV**) (i.e., post-stomach) appearance of EAA accounted for 106% of small intestinal disappearance in sheep fed at two levels of intake, but confirmed the apparent loss of EAA between the small intestine and portal vein when net PDV flux was measured. MacRae et al. (1997) suggested that use of arterial EAA by the stomach and hindgut may have accounted for the difference in net flux between the MDV and PDV. In a companion paper (Backwell et al., 1997), analysis of blood and plasma for peptides revealed no substantial peptide absorption across the MDV or PDV. These data suggest that release of peptides in portal blood may not account for lack of recovery of small intestinal protein disappearance. One additional factor that may contribute to low recoveries of amino acids is endogenous protein loss in the feces (MacRae et al., 1997). In our experiment, increasing tissue protein synthesis in the gut or endogenous protein loss in feces with increasing CP may account for the differences in PDV AAN recovery between 11.5C and 13.5UC.

Net Flux of Amino Acids and α-Amino N

An increase of 37.2 g/d in total N intake by casein infusion in heifers fed 11.5C elevated AAN and total amino acid N net appearance in portal blood by 43.1 and 221.8 g/d, respectively, in comparison with control heifers. Similarly, an increase of 37.2 g/d in total N intake by casein infusion in heifers fed 13.5UC elevated AAN and total amino acid N net appearance in portal blood by 18.9 and 106.2 g/d, respectively, compared with heifers fed 11.5U. Therefore, net rates of PDV absorption of total amino acids were fivefold greater than AAN flux. Inconsistencies between net amino acid and AAN flux have been shown previously (Reynolds et al., 1994; Bruckental et al., 1997). Reynolds et al. (1994) suggested that because the AAN chemistry used L-leucine as standard and the recoveries of other amino acids relative to L-leucine varies greatly (2 to 151%; Broderick and Kang, 1980), PA difference relative to the sum of PA difference for individual AA may have been reduced. This could have occurred in our experiment because L-leucine was used as the standard. In our experiment, net rates of PDV absorption of many amino acids were higher than previously reported for cattle (Huntington et al., 1988; Reynolds et al., 1994; Bruckental et al., 1997). In addition, glutamine was not separated under the conditions used in our amino acid analysis. Previous work with cattle fed high-concentrate diets (Prior et al., 1981; Bruckental et al., 1997) has shown negative net PDV fluxes of glutamine, albeit glutamine flux may be less negative when casein is infused (Bruckental et al., 1997). Because glutamine and glutamate together account for 24% of the amino acid composition of casein (Ling et al., 1961), utilization of glutamine by PDV tissues may have lowered the estimate of PDV flux of total amino acids when casein was infused in our experiment. However, it should be

noted that glutamine N taken up by rat intestine was accounted for as alanine (33%), citrulline (34%), ammonia (23%), and proline (10%; Windmueller and Spaeth, 1974). Therefore, if glutamine was removed by the PDV, a portion of the utilized glutamine N would be accounted for by increased PDV release of other amino acids.

Summary

Net PDV release of AAN was 125 and 83% greater when 11.5U and 13.5U were fed, respectively, compared with heifers fed 0% urea, suggesting that metabolizable protein supply to the small intestine was increased when .74% urea was fed, with no further improvement when 1.44% urea was fed. In addition, similar removal of urea N by the PDV with increasing urea suggests that urea was lost in the urine, resulting in inefficient use of N when 13.5U was fed. Feeding heifers 11.5C resulted in similar net PDV appearance of ammonia N and AAN and a greater arterial concentration of EAA compared with heifers fed 11.5U. However, the twofold greater removal of urea N by the PDV when 11.5C vs 11.5U was fed may not have been sufficient to meet the RDN requirement. When casein replaced urea and 13.5% CP was fed, AAN absorption across the PDV increased by 1.5-fold. Although the CP requirement was probably exceeded when heifers were fed 13.5% CP, providing N in the form of both RDN and escape protein increased N exchange across the PDV. This resulted from increases in appearance of ammonia N and AAN and a lower removal of urea N by the PDV compared with heifers fed 13.5% CP provided with urea. These data suggest that when the RDN requirement is met, escape protein must be supplied to enhance amino acid absorption.

Implications

For cattle consuming high-grain diets, an optimal amount of ruminally degraded nitrogen can be fed that will enhance fermentation in the rumen, increase amino acid flow to the duodenum, and increase net portal appearance of amino acids without affecting energy use by the portal-drained viscera. When the ruminally degraded nitrogen requirement has been met, additional ruminally degraded nitrogen will not be beneficial. Feeding ruminally degraded nitrogen and escape protein may increase amino acid absorption when compared with feeding ruminally degraded nitrogen alone at the same level of crude protein. However, exceeding the crude protein requirement may increase nitrogen excretion because of lower nitrogen recycling to the portal-drained viscera when ruminally degraded nitrogen and escape protein are fed.

Literature Cited

AOAC. 1990. Official Methods of Analysis (14th Ed.). Association of Official Analytical Chemists, Arlington, VA.

Backwell, F.R.C., M. Hipolito-Reis, D. Wilson, L. A. Bruce, V. Buchan, and J. C. MacRae. 1997. Quantification of circulating peptides and assessment of peptide uptake across the gastrointestinal tract of sheep. J. Anim. Sci. 75:3315–3322.

Bierman, S., T. Klopfenstein, R. Stock, and D. Shain. 1996. Evaluation of nitrogen, phosphorus, and organic matter balance in the feedlot as affected by nutrition. Nebraska Beef Cattle Rep. MP 66-A:74–77. Univ. of Nebraska, Lincoln.

Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63:64–75.

Bruckental, I., G. B. Huntington, C. Kirk Baer, and R. A. Erdman. 1997. The effect of abomasal infusion of casein and recombinant somatotropin hormone injection on nitrogen balance and amino acid fluxes in portal-drained viscera and net hepatic and total splanchnic blood in Holstein steers. J. Anim. Sci. 75:1119–1129.

Consortium. 1988. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, Champaign, IL.

Freetly, H. C., and C. L. Ferrell. 1998. Net flux of glucose, lactate, volatile fatty acids, and nitrogen metabolites across the portal-drained viscera and liver of pregnant ewes. J. Anim. Sci. 76: 3133–3145.

Gochmann, N., and J. M. Schmitz. 1972. Application of new peroxide indicator reaction to the specific, automated determination of glucose with glucose oxidase. Clin. Chem. 18:943–950.

Gregory, K. E., L. V. Cundiff, and R. M. Koch. 1991. Breed effects and heterosis in advanced generations of composite populations for growth traits in both sexes of beef cattle. J. Anim. Sci. 69: 3202–3212.

Guerino, F., G. B. Huntington, and R. A. Erdman. 1991. The net portal and hepatic flux of metabolites and oxygen consumption in growing beef steers given postruminal casein. J. Anim. Sci. 69:387–395.

Harvey, R. B., and A. J. Brothers. 1962. Renal extraction of para-aminohippurate and creatinine measured by continuous in vivo sampling of arterial and renal-vein blood. Ann. N.Y. Acad. Sci. 102:46–54.

Huntington, G. B. 1982. Portal blood flow and net absorption of ammonia-nitrogen, urea-nitrogen, and glucose in non-lactating Holstein cows. J. Dairy Sci. 65:1155–1162.

Huntington, G. B. 1986. Uptake and transfer of nonprotein nitrogen by the ruminant gut. Fed. Proc. 45:2272–2276.

Huntington, G. B. 1989. Hepatic urea synthesis and site and rate of urea removal from blood of beef steers fed alfalfa hay or a high concentrate diet. Can. J. Anim. Sci. 69:215–223.

Huntington, G. B., C. K. Reynolds, and B. H. Stroud. 1989. Techniques for measuring blood flow in splanchnic tissues of cattle. J. Dairy Sci. 72:1583–1595.

Huntington, G. B., G. A. Varga, B. P. Glenn, and D. R. Waldo. 1988. Net absorption and oxygen consumption by Holstein steers fed alfalfa or orchardgrass silage at two equalized intakes. J. Anim. Sci. 66:1292–1302.

Kennedy, P. M., and L. P. Milligan. 1980. The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants: A review. Can. J. Anim. Sci. 60:205–221.

Krehbiel, C. R., R. A. Britton, D. L. Harmon, J. P. Peters, R. A. Stock, and H. E. Grotjan. 1996. Effects of varying levels of duodenal or midjejunal glucose and 2-deoxyglucose infusion on small intestinal disappearance and net portal glucose flux in steers. J. Anim. Sci. 74:693–700.

Krehbiel, C. R., D. L. Harmon, and J. E. Schnieder. 1992. Effect of increasing ruminal butyrate on portal and hepatic nutrient flux in steers. J. Anim. Sci. 70:904–914.

Kreikemeier, K. K., C. L. Ferrell, H. C. Freetly, and A. L. Goetsch. 1994. Sheep, high grain diets, and ruminal escape protein. J. Anim. Sci. 72(Suppl. 2):91(Abstr.).

Larson, E. M., R. A. Stock, T. J. Klopfenstein, M. H. Sindt, and D. H. Shain. 1993. Energy value of hominy feed for finishing ruminants. J. Anim. Sci. 71:1092–1099.

Ling, E. R., S. K. Kon, and J.W.G. Porter. 1961. The composition of milk and the nutritive value of its components. In: S. K. Kon and A. T. Cowie (Ed.) Milk: The Mammary Gland and Its Secretion. Vol. II. pp 195–263. Academic Press, New York.

MacRae, J. C., L. A. Bruce, D. S. Brown, D.A.H. Farningham, and M. Franklin. 1997. Absorption of amino acids from the intestine and their net flux across the mesenteric- and portal-drained viscera of lambs. J. Anim. Sci. 75:3307–3314.

Mader, T., J. Dahlquist, K. Lechtenberg, and M. Thornsberry. 1995. Implant programs and melengestrol acetate (MGA) for weaned heifers placed in the feedlot. Nebraska Beef Cattle Rep. MP 62-A:43–45. Univ. of Nebraska, Lincoln.

Marsh, W. H., F. Benjamin, and H. Miller. 1965. Automated and manual direct methods for determination of blood urea. Clin. Chem. 11:624–627.

McDowell, R. E., P. C. Underwood, R. H. Lehmann, and M. S. Barrada. 1966. Procedure for exteriorizing the carotid artery in the bovine. J. Dairy Sci. 49:78–80.

Milton, C. T., R. T. Brandt, Jr., and E. C. Titgemeyer. 1997. Urea in dry-rolled corn diets: Finishing steer performance, nutrient digestion, and microbial protein production. J. Anim. Sci. 75: 1415–1424.

NRC. 1984. Nutrient Requirements of Beef Cattle (6th Ed.). National Academy Press, Washington, DC.

NRC. 1985. Ruminant Nitrogen Usage. National Academy Press, Washington, DC.

NRC. 1996. Nutrient Requirements of Beef Cattle (7th Ed.). National Academy Press, Washington, DC.

Palmer, D. W., and T. Peters, Jr. 1969. Automated determination of free amino groups in serum and plasma using 2, 4, 6 trinitrobenzene sulfonate. Clin. Chem. 15:891–901.

Prior, R. L., G. B. Huntington, and R. A. Britton. 1981. Influence of diet on amino acid absorption in beef cattle and sheep. J. Nutr. 111:2212–2222.

Reynolds, C. K., D. L. Harmon, R. L. Prior, and H. F. Tyrrell. 1994. Effects of mesenteric vein L-alanine infusion on liver metabolism of organic acids by beef heifers fed diets differing in forage:concentrate ratio. J. Anim. Sci. 72:3196–3206.

SAS. 1990. SAS User's Guide: Statistics (4th Ed.). SAS Inst. Inc., Cary, NC.

Windmueller, H. G., and A. E. Spaeth. 1974. Uptake and metabolism of plasma glutamine by the small intestine. J. Biol. Chem. 249:5070–5079.